Drug and Chemical Toxicology, 31:275–287, 2008

ISSN: 0148-0545 print / 1525-6014 online
DOI: 10.1080/01480540701873368

informa
healthcare

Lack of Gender-Related Difference in the Toxicity of 2-(2′-Hydroxy-3′,5′-di-*tert*-butylphenyl)benzotriazole in Preweaning Rats

Mutsuko Hirata-Koizumi,[1] Takashi Matsuyama,[2] Toshio Imai,[1] Akihiko Hirose,[1] Eiichi Kamata,[1] and Makoto Ema[1]

[1]Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan
[2]Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories, Ltd. (SNBL DSR), Kagoshima, Japan

In our previous toxicity studies using young rats, we showed that an ultraviolet absorber, 2-(2′-hydroxy-3′,5′-di-*tert*-butylphenyl)benzotriazole (HDBB), principally affected the liver, and male rats had nearly 25 times higher susceptibility to the toxic effects than females. In the present study, the toxicity of HDBB was investigated in preweaning rats. HDBB was administered by gavage to male and female CD(SD) rats from postnatal days 4 to 21 at a dose of 0, 0.1, 0.5, 2.5, or 12.5 mg/kg/day. No substance-related deaths, clinical signs of toxicity, or body-weight changes were observed. Increased levels of albumin, AST and ALP in both sexes, BUN in males, and LDH in females were found at 12.5 mg/kg. Liver weights increased at 2.5 mg/kg and above in both sexes. Histopathologically, hepatocellular findings, such as nucleolar enlargement, anisokaryosis, increased mitosis, and/or hypertrophy, were observed at 2.5 mg/kg and above in both sexes. These results indicate no gender-related differences in the susceptibility to the toxic effects of HDBB in preweaning rats.

Keywords Benzotriazole UV absorber, Preweaning rat, Gender-related difference, Hepatotoxicity.

Address correspondence to Makoto Ema, Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan; E-mail: ema@nihs.go.jp

INTRODUCTION

A number of reports have been published on gender-related differences in the toxic effects of chemicals in rats (Agarwal et al., 1982; Coleman et al., 1990; McGovren et al., 1981; Muraoka and Itoh, 1980; Nishino et al., 1998; Ogirima et al., 2006; Raheja et al., 1983). For example, fluoranthene, a polycyclic aromatic hydrocarbon, showed greater effects on male rats than females, especially on the kidneys, in a subchronic toxicity study (Knuckles et al., 2004). In contrast, female rats exhibited greater susceptibility to hypothalamic cholinesterase inhibitory and hypothermic effects of a carbamate cholinesterase inhibitor, rivastigmine (Wang et al., 2001). Such gender-related variations are also reported in humans, mostly for medicines (Harris et al., 1995). Examples include more severe adverse effects, but with greater improvement in response, to antipsychotic drugs such as chlorpromazine and fluspirilene in women.

Previously, we reported that male and female rats showed markedly different susceptibilities to the toxicity of 2-(2′-hydroxy-3′,5′-di-*tert*-butylphenyl)benzotriazole (HDBB), which is an ultraviolet absorber used in plastic resin products, such as building materials and automobile components (METI, 2006). In a 28-day repeated-dose toxicity study, male and female rats were administered HDBB by gavage, and adverse effects on the liver, heart, blood, kidneys, and thyroids were found (Hirata-Koizumi et al., 2007). The no observed adverse effect level (NOAEL) for females was 2.5 mg/kg/day based on histopathological changes in the liver and heart detected at 12.5 mg/kg, but the NOAEL for males could not be determined because hepatic changes were noted even at the lowest dose of 0.5 mg/kg. In the 52-week repeated-dose toxicity study, chronic oral administration of HDBB principally affected the liver, and the NOAEL was concluded to be 0.1 mg/kg/day in males and 2.5 mg/kg/day in females (Hirata-Koizumi et al., 2008a), showing that male rats have approximately 25 times higher susceptibility to HDBB toxicity than females.

For such gender differences in toxic responses, sexual hormones are likely to play important roles. In fact, Wang et al. (2001) reported that orchidectomy completely abolished the above-mentioned sex differences in hypothalamic cholinesterase inhibition induced by rivastigmine, and testosterone treatment to gonadectomized males and females decreased the cholinesterase inhibitory effects of rivastigmine; therefore, it is apparent that testosterone interferes with the effects of rivastigmine. On the other hand, estrogen has been shown to act as a dopamine antagonist (Harris et al., 1995), which is considered to contribute, at least in part, to sex differences in response to antipsychotic drugs.

In order to investigate the role of sex steroids in the mediation of sex differences in the susceptibility to the toxic effects of HDBB, we recently performed a 28-day repeated-dose toxicity study using male and female

castrated rats (Hirata-Koizumi et al., 2008b). As expected, castration markedly reduced the sexual variation in HDBB toxicity, but some difference, less than five times, remained between male and female castrated rats. It is speculated that the determinants of susceptibility to HDBB toxicity are already differentiated between sexes by four weeks of age, when the castration was performed; therefore, in the present study, we determined the sexual difference in the susceptibility to HDBB toxicity in preweaning rats.

MATERIALS AND METHODS

This study was performed at Shin Nippon Biomedical Laboratories, Ltd., Drug Safety Research Laboratories (SNBL DSR; Kagoshima, Japan) in 2006–2007. The experiment was approved by the Institutional Animal Care and Use Committee of SNBL DSR and was performed in accordance with the ethics criteria contained in the bylaws of the Committee.

Animals and Housing Conditions

Eleven-week-old male and 10-week-old female Crl:CD(SD) rats were purchased from Hino Breeding Center, Charles River Laboratories Japan, Inc. (Yokohama, Japan) and individually housed in stainless steel cages suspended over a cage board. After a seven-day acclimation, females were cohabited overnight with one male each. Females with vaginal plugs were regarded as pregnant, and this day was designated as Day 0 of gestation. On gestation day 20, the pregnant females were transferred to aluminum cages with wooden chips as bedding (White Flake; Charles River Laboratories Japan, Inc.) and allowed to deliver spontaneously and rear their pups. The day of birth was defined as postnatal day (PND) 0. On PND 4, the sex of the pups was determined, and the litters were adjusted randomly to four males and four females. Five litters were selected and randomly assigned to each of five dose groups, including control groups; the initial number of pups for treatment was 20/sex/group.

Throughout the study, the animals were maintained in an air-conditioned room at 21.5–22.4°C, with a relative humidity of 43–55%, a 12-h light/dark cycle, and ventilation with 15 air changes/hour. A basal diet (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water, which met the drinking water standard under the Water Works Law of Japan, were provided *ad libitum*.

Chemicals and Doses

HDBB (CAS No. 3846-71-7, Lot no. AY11) was 100% pure and was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); it was kept in a dark place at room temperature under airtight conditions. Dosing

solutions were prepared as a suspension in corn oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan) once or twice a week and kept cool in a dark place under airtight conditions until dosing. Stability under refrigerated conditions was confirmed for seven days in the previous 28-day repeated-dose toxicity study using young animals (Hirata-Koizumi et al., 2007).

Male and female preweaning rats were given HDBB by gavage once-daily from PNDs 4 to 21. Control rats received the vehicle only. A nutrient catheter (Type 3Fr; Atom Medical Corporation, Tokyo, Japan), attached to a disposable syringe, was used for dosing. The volume of each dose was adjusted to 10 mL/kg of body weight, based on the latest body weight.

The dosage levels of HDBB were determined to be 0.1, 0.5, 2.5, or 12.5 mg/kg/day, based on the results of our previous 28-day repeated-dose toxicity study using young rats (Hirata-Koizumi et al., 2007). In this previous study, male and female young rats were given HDBB by gavage at 0.5, 2.5, 12.5, or 62.5 mg/kg/day, and adverse effects, mainly on the liver and heart, were found at all doses in males and at 12.5 mg/kg and above in females.

Observations

All dams were observed daily for clinical signs of toxicity, and body weight was recorded on Days 0, 10, and 20 of pregnancy and on Days 0, 10, 20, and 22 after delivery. On Day 22 after delivery, they were euthanized by exsanguination under deep ether anesthesia, and the surface, organs, and tissues of the entire body were macroscopically observed.

All pups were observed daily before and three to four hours after dosing for clinical signs of toxicity. Body weight was recorded on PNDs 0, 4, 6, 8, 10, 12, 14, 16, 18, 21, and 22. On PND 22, blood was collected from the caudal vena cava in the abdomen of two male and two female pups per litter under deep ether anesthesia. Plasma separated from the blood by centrifugation was examined for total protein, albumin, glucose, total cholesterol, triglycerides, total bilirubin, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine phosphokinase, calcium, inorganic phosphorus, sodium, potassium, and chlorine. Following the collection of blood, all pups (four males and four females per litter) were euthanized by exsanguination under deep ether anesthesia, and the surface, organs, and tissues of the entire body were macroscopically observed. The heart, lungs, liver, spleen, kidneys, and adrenals were then collected and weighed. The liver and heart were histopathologically examined in one male and one female per litter. The organs were fixed in 10% neutral-buffered formalin, and paraffin sections for microscopic examination were routinely prepared and stained with hematoxylin-eosin.

Data Analysis

Body weight, blood biochemical parameters, and organ weights of pups were analyzed by Bartlett's test (Bartlett, 1937) for homogeneity of distribution ($p < 0.01$). When homogeneity was recognized, Dunnett's test (Dunnett, 1964) was conducted to compare between control and individual treatment groups ($p < 0.01$ or 0.05). If not homogenous, data were analyzed using the mean rank test of Dunnett's type (Hollander and Wolfe, 1973) ($p < 0.01$ or 0.05). Histopathological findings were analyzed using Wilcoxon's rank sum test (Wilcoxon, 1945) ($p < 0.01$ or 0.05).

RESULTS

HDBB, orally administered to pups from PNDs 4 to 21, did not induce any clinical signs of toxicity or affect the body weight of maternal rats (data not shown). At necropsy, no gross abnormality was found in the dams.

One male pup each at 0 or 0.5 mg/kg and one female pup each at 0, 0.5, or 12.5 mg/kg died, which was confirmed to be due to gavage error. No substance-related clinical signs of toxicity were found in pups of any groups. There were also no significant changes in the body weight of male and female pups, as shown in Figure 1.

Principle blood biochemical values are summarized in Table 1. In males, the levels of albumin, AST, ALP, and BUN were significantly increased at 12.5 mg/kg. In females, significant increases in the levels of albumin, AST, ALP, and LDH were found at the same dose. There were no substance-related changes in other blood biochemical parameters.

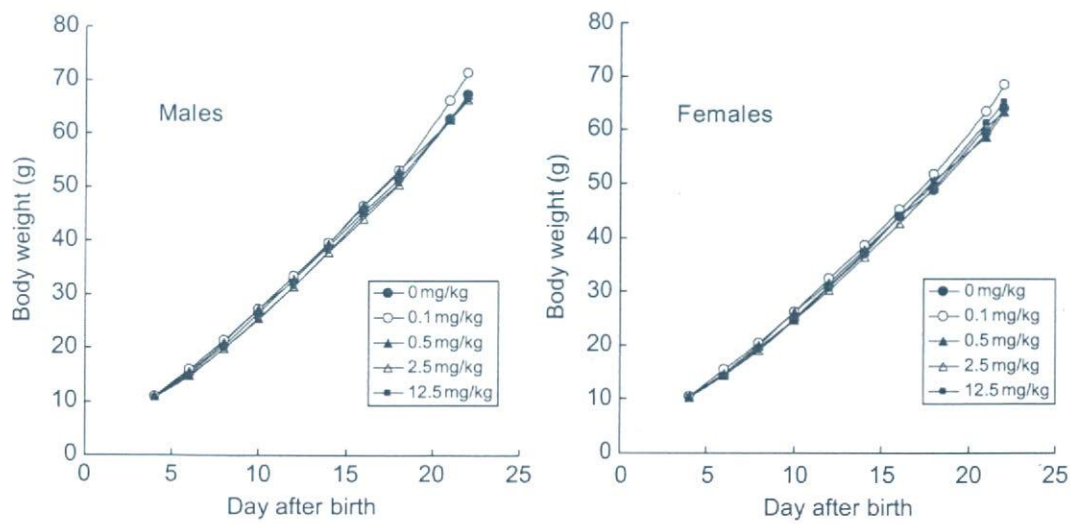


Figure 1: Body weight curves of male and female preweaning rats given HDBB by gavage.

Table 1: Principle blood biochemical values in male and female preweaning rats given HDBB by gavage.

Dose (mg/kg/day)	0	0.1	0.5	2.5	12.5
No. of males	10	10	10	10	10
Total protein (g/dL)	4.49 ± 0.28	4.53 ± 0.22	4.48 ± 0.26	4.43 ± 0.17	4.42 ± 0.18
Albumin (g/dL)	3.62 ± 0.24	3.60 ± 0.24	3.59 ± 0.21	3.74 ± 0.27	4.04 ± 0.17**
BUN (mg/dL)	11.4 ± 1.5	14.1 ± 2.6	13.7 ± 5.3	12.9 ± 1.8	14.7 ± 2.3**
AST (IU/L)	91.4 ± 15.9	85.2 ± 4.8	88.7 ± 5.2	91.6 ± 12.2	100.2 ± 8.5*
ALT (IU/L)	34.8 ± 5.7	34.0 ± 6.3	29.4 ± 5.3	30.7 ± 5.5	35.9 ± 6.1
ALP (IU/L)	1557 ± 203	1529 ± 240	1412 ± 279	1286 ± 249	2054 ± 444**
LDH (IU/L)	198 ± 123	165 ± 16	184 ± 40	236 ± 170	326 ± 221
No. of females	10	10	10	10	10
Total protein (g/dL)	4.49 ± 0.24	4.54 ± 0.24	4.53 ± 0.28	4.55 ± 0.18	4.50 ± 0.14
Albumin (g/dL)	3.59 ± 0.28	3.66 ± 0.24	3.70 ± 0.26	3.80 ± 0.25	4.04 ± 0.16**
BUN (mg/dL)	12.5 ± 2.0	15.4 ± 1.5	13.5 ± 4.0	14.1 ± 4.1	15.5 ± 3.3
AST (IU/L)	87.3 ± 9.4	85.1 ± 8.2	86.5 ± 6.3	85.2 ± 6.6	101.3 ± 9.2**
ALT (IU/L)	30.7 ± 5.9	30.7 ± 3.6	27.1 ± 5.5	27.1 ± 4.5	35.9 ± 4.2
ALP (IU/L)	1470 ± 136	1394 ± 215	1287 ± 105	1339 ± 183	1872 ± 259**
LDH (IU/L)	175 ± 52	176 ± 36	179 ± 35	139 ± 28	370 ± 295*

Values are expressed as the mean ± SD.
BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.
*Significantly different from the control group ($p < 0.05$).
**Significantly different from the control group ($p < 0.01$).

At necropsy, no gross abnormality was observed. Absolute and relative organ weights of scheduled sacrifice animals are shown in Table 2. In males, absolute liver weight at 12.5 mg/kg and relative weight at 2.5 mg/kg and above were significantly increased. In addition, absolute and relative weights of the lungs and spleen were significantly decreased at 12.5 mg/kg. In females, significant increases in absolute liver weight at 12.5 mg/kg and relative liver weight at 2.5 mg/kg and above, and decreases in relative spleen weight and absolute and relative adrenal weight at 12.5 mg/kg, were found. No substance-related changes were detected in other organ weights.

Histopathological findings in the liver are presented in Table 3. In males, nucleolar enlargement, anisokaryosis, and increased mitosis of hepatocytes were observed at 2.5 mg/kg and above. In the 12.5 mg/kg group, hypertrophy of hepatocytes accompanied with eosinophilic granular changes was also observed. Further, increased incidence and/or severity of decreased glycogen in hepatocytes was found at 2.5 mg/kg and above. Similarly, in females, nucleolar enlargement, anisokaryosis, and increased mitosis of hepatocytes at 2.5 mg/kg and above, and hypertrophy and eosinophilic granular change of hepatocytes at 12.5 mg/kg were detected, and the incidence and/or severity of decreased glycogen in hepatocytes was higher at 12.5 mg/kg. No substance-related histopathological changes were detected in the heart in both sexes.

Table 2: Organ weights of male and female preweaning rats given HDBB by gavage.

Dose (mg/kg/day)	0	0.1	0.5	2.5	12.5
No. of males	19	20	19	20	20
Body weight (g)	67.2 ± 7.3	71.3 ± 6.9	67.3 ± 5.8	66.2 ± 9.6	66.2 ± 5.0
Heart (g)	0.37 ± 0.04	0.37 ± 0.04	0.36 ± 0.05	0.36 ± 0.05	0.35 ± 0.04
	(0.55 ± 0.04)	(0.52 ± 0.04)	(0.53 ± 0.05)	(0.54 ± 0.03)	(0.53 ± 0.04)
Lung (g)	0.58 ± 0.07	0.58 ± 0.04	0.53 ± 0.03*	0.59 ± 0.08	0.53 ± 0.04*
	(0.87 ± 0.07)	(0.82 ± 0.09)	(0.80 ± 0.06*)	(0.90 ± 0.09)	(0.80 ± 0.06*)
Liver (g)	2.83 ± 0.47	2.88 ± 0.34	2.75 ± 0.44	3.24 ± 0.68	4.54 ± 0.61**
	(4.19 ± 0.36)	(4.04 ± 0.26)	(4.07 ± 0.42)	(4.87 ± 0.40**)	(6.84 ± 0.53**)
Spleen (g)	0.37 ± 0.09	0.40 ± 0.05	0.34 ± 0.08	0.38 ± 0.77	0.29 ± 0.05**
	(0.55 ± 0.10)	(0.57 ± 0.06)	(0.51 ± 0.10)	(0.57 ± 0.08)	(0.44 ± 0.06**)
Kidneys (g)	0.72 ± 0.09	0.74 ± 0.06	0.72 ± 0.08	0.68 ± 0.10	0.71 ± 0.07
	(1.07 ± 0.07)	(1.04 ± 0.07)	(1.07 ± 0.08)	(1.03 ± 0.05)	(1.07 ± 0.08)
Adrenals (mg)	17.5 ± 3.7	19.3 ± 3.7	18.1 ± 3.3	21.5 ± 5.2*	17.0 ± 2.4
	(26.2 ± 5.1)	(27.3 ± 5.8)	(27.4 ± 5.8)	(32.4 ± 6.8**)	(25.6 ± 3.3)
No. of females	19	20	19	20	19
Body weight (g)	64.0 ± 7.1	68.6 ± 7.5	63.6 ± 4.7	63.6 ± 8.9	65.3 ± 4.1
Heart (g)	0.35 ± 0.05	0.35 ± 0.05	0.33 ± 0.03	0.34 ± 0.05	0.35 ± 0.04
	(0.54 ± 0.04)	(0.51 ± 0.05)	(0.52 ± 0.06)	(0.53 ± 0.04)	(0.53 ± 0.04)
Lung (g)	0.54 ± 0.08	0.54 ± 0.06	0.55 ± 0.06	0.57 ± 0.09	0.51 ± 0.05
	(0.85 ± 0.11)	(0.80 ± 0.09)	(0.86 ± 0.10)	(0.90 ± 0.12)	(0.78 ± 0.06)
Liver (g)	2.72 ± 0.47	2.77 ± 0.41	2.62 ± 0.38	3.01 ± 0.54	4.47 ± 0.39**
	(4.23 ± 0.43)	(4.02 ± 0.24)	(4.12 ± 0.44)	(4.71 ± 0.27*)	(6.84 ± 0.41**)
Spleen (g)	0.36 ± 0.12	0.37 ± 0.06	0.32 ± 0.07	0.33 ± 0.06	0.28 ± 0.07
	(0.55 ± 0.15)	(0.53 ± 0.07)	(0.50 ± 0.10)	(0.52 ± 0.08)	(0.43 ± 0.09*)
Kidneys (g)	0.70 ± 0.07	0.71 ± 0.07	0.67 ± 0.06	0.66 ± 0.09	0.72 ± 0.07
	(1.09 ± 0.05)	(1.04 ± 0.04**)	(1.05 ± 0.05)	(1.04 ± 0.05*)	(1.10 ± 0.07)
Adrenals (mg)	19.2 ± 3.7	18.8 ± 4.5	16.9 ± 2.3	19.9 ± 3.7	15.4 ± 3.5**
	(29.9 ± 4.6)	(27.5 ± 6.8)	(26.8 ± 4.2)	(31.4 ± 5.2)	(23.5 ± 4.8**)

Values are expressed as the mean ± SD.
Values in parentheses are relative organ weights (g or mg/100 g body weight).
*Significantly different from the control group ($p < 0.05$).
**Significantly different from the control group ($p < 0.01$).

Table 3: Histopathological findings in the liver of male and female preweaning rats given HDBB by gavage.

		Dose (mg/kg/day)					
	Grade	0	0.1	0.5	2.5	12.5	
No. of males		5	5	5	5	5	
Nucleolar enlargement in hepatocytes	±	0	0	0	1	4	**
	+	0	0	0	0	1	
Anisokaryosis of hepatocytes	±	0	0	0	1	2	**
	+	0	0	0	0	3	
Increased mitosis of hepatocytes	±	0	1	0	2	1	**
	+	0	0	0	1	3	
	++	0	0	0	0	1	
Hypertrophy of hepatocytes	±	0	0	0	0	4	**
	+	0	0	0	0	1	
Eosinophilic granular change of hepatocytes	+	0	0	0	0	5**	
Decreased glycogen in hepatocytes	±	1	1	2	4	2	*
	+	0	0	0	0	3	
No. of females		5	5	5	5	5	
Nucleolar enlargement in hepatocytes	±	0	0	0	2	4	**
	+	0	0	0	0	1	
Anisokaryosis of hepatocytes	±	0	0	0	1	3	**
	+	0	0	0	0	2	
Increased mitosis of hepatocytes	±	0	1	0	1	1	**
	+	0	0	0	2	3	
	++	0	0	0	0	1	
Hypertrophy of hepatocytes	±	0	0	0	0	3	**
	+	0	0	0	0	2	
Eosinophilic granular change of hepatocytes	±	0	0	0	0	1	**
	+	0	0	0	0	4	
Decreased glycogen in hepatocytes	±	1	0	2	2	3	*
	+	0	0	0	0	2	

Values represent the number of animals with the finding.
±, very slight; +, slight; ++, moderate.
*Significantly different from the control ($p < 0.05$).
**Significantly different from the control ($p < 0.01$).

DISCUSSION

In the current study, the toxicity of HDBB was investigated in preweaning rats. Based on our previous results of a 28-day repeated-dose toxicity study using young rats (Hirata-Koizumi et al., 2008a), the dosage of HDBB used in this study was sufficiently high to be expected to induce adverse effects on the liver and heart. As expected, increased absolute and/or relative liver weight and histopathological changes of hepatocytes were observed at 2.5 mg/kg and above in both sexes.

Although degeneration and hypertrophy of the myocardium or cell infiltration in the heart were observed at 2.5 mg/kg and above in the previous 28-day study (Hirata-Koizumi et al., 2007), such changes were not detected even at the highest dose of 12.5 mg/kg in the present study. Considering that histopathological changes in the heart were also not found in the previous 52-week study of HDBB using young rats (Hirata-Koizumi et al., 2008a) and a 28-day study using young castrated rats (Hirata-Koizumi et al., 2008b), it could not be concluded that preweaning rats were less susceptible to the cardiac effects of HDBB than young rats. In order to investigate the toxicological effects of HDBB on the heart in more detail, the effects on cardiac function (e.g., electrocardiographic parameters, blood pressure, etc.) should be evaluated because they are considered to be more susceptible parameters than histopathology of the heart (Glaister, 1992).

In the present study, some blood biochemical parameters increased in both sexes in the 12.5 mg/kg group. The degree of change was mostly slight, but it was considered to be HDBB related because similar changes were found in previous studies of HDBB (Hirata-Koizumi et al., 2007, 2008a, 2008b). A simultaneous increase in hepatic enzymes (AST, ALP, and LDH) might result from hepatic damage caused by HDBB. Increased BUN suggests renal effects of HDBB, although histopathology of the kidneys was not examined in the present study. As a matter of fact, hypertrophy of the tubular epithelium was noted at 12.5 mg/kg and above in males and at 62.5 mg/kg in females in the previous 28-day study of HDBB using young rats (Hirata-Koizumi et al., 2007).

No effects on the lungs, spleen, and adrenals were found both in previous 28-day and 52-week studies of HDBB using young rats (Hirata-Koizumi et al., 2007, 2008a), whereas decreased weight of these organs was found in preweaning rats given HDBB. In rats, many organs develop rapidly during the early period after birth (Vidair, 2004; Walthall et al., 2005; Zoetis and Hurtt, 2005a). For example, rat lungs have no alveoli at birth, but they develop rapidly, with most lung development complete within the first two weeks after birth (Zoetis and Hurtt, 2005b). It is conceivable that immature and/or rapidly developing organs show different susceptibility from mature organs. Considering these findings together suggests that HDBB might influence these organs, specifically in the preweaning period. Further studies are required to investigate the adverse effects of HDBB on the lungs, spleen, and adrenals during the preweaning period.

Histopathological changes in the liver detected in the current study included nucleolar enlargement, anisokaryosis, increased mitosis, and hypertrophy of hepatocytes. Nucleolar enlargement of hepatocytes indicates the enhancement of protein synthesis and is identified most frequently in hepatocytes that are undergoing rapid cell proliferation (Cattley and Popp, 2002). Anisokaryosis is also considered to correlate at least partly with cell

proliferation. In the present study, nucleolar enlargement, anisokaryosis, and increased mitosis of hepatocytes were observed at 2.5 mg/kg and above in both sexes, whereas hypertrophy of hepatocytes was observed only at the highest dose of 12.5 mg/kg. On the other hand, in the previous 28-day study of HDBB using young rats, hypertrophy of hepatocytes was observed at 0.5 mg/kg and above in males and 12.5 mg/kg and above in females, and increased mitosis of hepatocytes was observed at 62.5 mg/kg and 12.5 mg/kg and above in males and females, respectively, indicating that young rats are more susceptible to the HDBB-induced hypertrophic response of hepatocytes than the mitotic response (Hirata-Koizumi et al., 2007). The higher susceptibility of preweaning rats to such proliferative changes might be associated with dramatic changes of the liver structure during the preweaning period (Alexander et al., 1997).

In previous studies using young rats (five to six weeks of age), we showed that male rats were much more susceptible to the toxic effects of HDBB than females (Hirata-Koizumi et al., 2007, 2008a). Based on histopathological findings in the liver, which is a major target of HDBB toxicity, differences in susceptibility between sexes was approximately 25 times. Subsequently, we showed that castration markedly reduced the gender-related differences in HDBB hepatotoxicity in rats (Hirata-Koizumi et al., 2008b). Comparing the histopathological findings of the liver observed in the previous 28-day studies using young intact and castrated rats, it became clear that the castration of male rats exerted no effect but that of female rats enhanced the adverse effects of HDBB on the liver, suggesting suppressive effects of estrogen on the hepatotoxicity of HDBB in rats. Despite the marked reduction of gender-related differences in the toxic effects of HDBB by castration, a difference, less than five times, remained in castrated rats. The sexual differences in castrated rats are considered to be due to the exposure to sexual hormones before four weeks of age, when castration was conducted. In the present study, following the administration of HDBB during the preweaning period, similar changes in all examined parameters were observed at the same doses in both sexes. These findings clearly show no gender-related differences in HDBB toxicity in preweaning rats, suggesting that a development at around three to six weeks of age contributes to sexual variations in HDBB toxicity, at least in part.

Gender-related differences in HDBB toxicity were found not only for hepatotoxicity, but also for the reduction of body weight, hematotoxicity, cardiac toxicity, etc., in the previous 28-day and/or 52-week studies using young rats (Hirata-Koizumi et al., 2007, 2008a). Thus, they might be caused by differences in the blood concentration of causative substances (e.g., HDBB or its metabolites) between sexes. A number of reports have been published on the sexual variations in toxicokinetic determinants, such as hepatic metabolism (Gad, 2006) and membrane transporter in various organs, including the kidneys and intestine (Morris et al., 2003). Coleman et al. (1990) reported that

higher sensitivity of male rats to hematotoxicity of dapsone, which is a major component of the multidrug regimen for the treatment of leprosy, was due to the greater capacity for the N-hydroxylation. Another example was an amino acid antitumor agent, acivicin, of which the LD_{50} was much higher in male mice than that in females. McGovren et al. (1981) showed that the plasma half-time was much longer in female mice and speculated that the sexual variation may be related to differences in the renal excretion.

For gender-related differences in toxicokinetic determinants, many mechanistic studies have been reported on the metabolic enzyme cytochrome P450 (CYP) (Waxman and Chang, 2005). In rats, a subset of CYPs is expressed in a sex-dependent fashion. It was reported that ovariectomy reduced the hepatic expression of female-specific/predominant CYPs, but this did not lead to the expression of male-specific CYP enzyme in female rats. If female-specific/predominant metabolic enzymes have an intimate involvement in the detoxication of HDBB, our previous results, showing the higher susceptibility of male young rats to HDBB toxicity than females, and increased susceptibility by castration of females, could be explained. Interestingly, in rat liver, the difference in CYP expression between sexes is not apparent until puberty (Waxman and Chang, 2005). This is consistent with our present results that there was no gender-related difference in HDBB hepatotoxicity in preweaning rats. Mode and Gustafsson (2006) reported that brain centers involved in the hypothalamo-pituitary control of hepatic sex-dependent metabolism in adults are irreversibly programmed by neonatal androgen exposure, which might explain why sexual variation in HDBB toxicity was not completely abolished by castration at four weeks of age.

In order to clarify the cause of gender differences, we are currently performing a toxicokinetic study of HDBB, which includes the identification of metabolites and the related metabolic enzyme as well as measurement of the blood concentration of HDBB both after single and repeated administration of HDBB to young and preweaning rats.

CONCLUSION

The current results showed that oral administration of HDBB to preweaning rats caused hepatotoxicity at 2.5 mg/kg and above in both sexes. The gender-related difference in toxic susceptibility to HDBB, which was observed in young rats, was not detected in preweaning rats.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Health, Labour and Welfare, Tokyo, Japan.

REFERENCES

Agarwal, D. K., Misra, D., Agarwal, S., Seth, P. K., Kohli, J. D. (1982). Influence of sex hormones on parathion toxicity in rats: antiacetylcholinesterase activity of parathion and paraoxon in plasma, erythrocytes, and brain. *J. Toxicol. Environ. Health.* 9:451–459.

Alexander, B., Guzail, M. A., Foster, C. S. (1997). Morphological changes during hepatocellular maturity in neonatal rats. *Anat. Rec.* 248:104–109.

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proc. R. Soc. Lond. Ser. A* 160:268–282.

Cattley, R. C., Popp, J. A. (2002). Liver: In: Haschek, W. M., Rousseaux, C. G., Wallig, M. A., eds. *Handbook of Toxicologic Pathology, 2nd ed.,* Vol. 2. San Diego, California, USA: Academic Press, pp. 187–225.

Coleman, M. D., Tingle, M. D., Winn, M. J., Park, B. K. (1990). Gonadal influence on the metabolism and haematological toxicity of dapsone in the rat. *J. Pharm. Pharmacol.* 42:698–703.

Coleman, M. D., Winn, M. J., Breckenridge, A. M., Park, B. K. (1990). Sex-dependent sensitivity to dapsone-induced methaemoglobinaemia in the rat. *Biochem. Pharmacol.* 39:805–809.

Dunnett, C. W. (1964). New tables for multiple comparisons with a control. *Biometrics* 20:482–491.

Gad, S. C. (2006). Metabolism. In: Gad, S. C., ed. *Animal Models in Toxicology, 2nd ed.* Boca Raton, Florida, USA: CRC Press, pp. 217–247.

Glaister, J. R. (1992). Histopathology of target organs—cardiovascular: In: *Principles of Toxicological Pathology (Japanese version supervised by Takahashi, M.).* Tokyo: Soft Science Inc., pp.135–142.

Harris, R. Z., Benet, L. Z., Schwartz, J. B. (1995). Gender effects in pharmacokinetics and pharmacodynamics. *Drug*s 50:222–239.

Hirata-Koizumi, M., Watari, N., Mukai, D., Imai, T., Hirose, A., Kamata, E., Ema, M. (2007). A 28-day repeated dose toxicity study of ultraviolet absorber 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole in rats. *Drug Chem. Toxicol.* 30: 327–341.

Hirata-Koizumi, M., Ogata, H., Imai, T., Hirose, A., Kamata, E., Ema, M. (2008a). A 52-week repeated dose toxicity study of ultraviolet absorber 2-(2'-hydroxy-3', 5'-di-*tert*-butylphenyl)benzotriazole in rats. *Drug Chem. Toxicol.* 31:81–96.

Hirata-Koizumi, M., Matsuyama, T., Imai, T., Hirose, A., Kamata, E., Ema, M. (2008b). Gonadal influence on the toxicity of 2-(2′-hydroxy-3′,5′-di-*tert*-butylphenyl) benzotriazole in rats. *Drug Chem. Toxicol.* 31:115–126.

Hollander, M., Wolfe, D. A. (1973). *Nonparametric Statistical Methods.* New York: John Wiley and Sons.

Knuckles, M. E., Inyang, F., Ramesh, A. (2004). Acute and subchronic oral toxicity of fluoranthene in F-344 rats. *Ecotoxicol. Environ. Saf.* 59:102–108.

McGovren, J. P., Neil, G. L., Chan, P. J., Stewart, J. C. (1981). Sex- and age-related mouse toxicity and disposition of the amino acid antitumor agent, acivicin. *J. Pharmacol. Exp. Ther.* 216:433–440.

METI (Ministry of Economy, Trade and Industry of Japan). (2006). 2-(2H-1,2,3-benzotriazole-2-yl)-4,6-di-*tert*-butylphenol (In Japanese), document distributed in Committee on Safety of Chemical Substances, Chemical

Substances Council, 30 June 2006. Available at: http://www.meti.go.jp/committee/materials/g60705aj.html Accessed on September 19, 2007.

Mode, A., Gustafsson, J. A. (2006). Sex and the liver—a journey through five decades. *Drug Metab. Rev.* 38:197–207.

Morris, M. E., Lee, H. J., Predko, L. M. (2003). Gender differences in the membrane transport of endogenous and exogenous compounds. *Pharmacol. Rev.* 55:229–240.

Muraoka, Y., Itoh, F. (1980). Sex difference of mercuric chloride-induced renal tubular necrosis in rats—from the aspect of sex differences in renal mercury concentration and sulfhydryl levels. *J. Toxicol. Sci.* 5:203–214.

Nishino, H., Nakajima, K., Kumazaki, M., Fukuda, A., Muramatsu, K., Deshpande, S. B., Inubushi, T., Morikawa, S., Borlongan, C. V., Sanberg, P. R. (1998). Estrogen protects against while testosterone exacerbates vulnerability of the lateral striatal artery to chemical hypoxia by 3-nitropropionic acid. *Neurosci. Res.* 30:303–312.

Ogirima, T., Tano, K., Kanehara, M., Gao, M., Wang, X., Guo, Y., Zhang, Y., Guo, L., Ishida, T. (2006). Sex difference of adenine effects in rats: renal function, bone mineral density, and sex steroidogenesis. *Endocr. J.* 53:407–413.

Raheja, K. L., Linscheer, W. G., Cho, C. (1983). Hepatotoxicity and metabolism of acetaminophen in male and female rats. *J. Toxicol. Environ. Health.* 12:143–158.

Vidair, C. A. (2004). Age dependence of organophosphate and carbamate neurotoxicity in the postnatal rat: extrapolation to the human. *Toxicol. Appl. Pharmacol.* 196:287–302.

Walthall, K., Cappon, G. D., Hurtt, M. E., Zoetis, T. (2005). Postnatal development of the gastrointestinal system: a species comparison. *Birth Defects Res. B Dev. Reprod. Toxicol.* 74:132–156.

Wang, R. H., Schorer-Apelbaum, D., Weinstock, M. (2001). Testosterone mediates sex difference in hypothermia and cholinesterase inhibition by rivastigmine. *Eur. J. Pharmacol.* 433:73–79.

Waxman, D. J., Chang, T. K. (2005). Hormonal regulation of liver cytochrome P450 enzymes. In: Ortiz de Montellano, P. R., ed. *Cytochrome P450—Structure, Mechanism, and Biochmistry, 3rd ed.*New York: Kluwer Academic/ Plenum Publishers, pp. 347–376

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bull.* 1:80–83.

Zoetis, T., Hurtt, M. E. (2005a) Species comparison of anatomical and functional renal development. *Birth Defects Res. B Dev. Reprod. Toxicol.* 68:111–120.

Zoetis, T., Hurtt, M. E. (2005b) Species comparison of lung development. *Birth Defects Res. B Dev. Reprod. Toxicol.* 68:121–124.

Reproductive Toxicology 26 (2008) 185–190

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Review

Review of testicular toxicity of dinitrophenolic compounds, 2-*sec*-butyl-4,6-dinitrophenol, 4,6-dinitro-*o*-cresol and 2,4-dinitrophenol

Mariko Matsumoto*, Akihiko Hirose, Makoto Ema

Division of Risk Assessment, Biological Safety Center, National Institute of Health Sciences, 1-18-1 kamiyoga, Tokyo 185-8501, Japan

ARTICLE INFO

Article history:
Received 11 June 2008
Received in revised form 4 September 2008
Accepted 24 September 2008
Available online 7 October 2008

Keywords:
Dinitrophenols
Testicular toxicity
Spermatotoxicity
Male reproductive toxicity
Dinoseb
DNP
DNOC

ABSTRACT

The present review paper summarizes the data available in the literature concerning dinitrophenolic compounds and evaluates male reproductive toxicity in experimental animals. Gavage and feeding doses of 2-*sec*-butyl-4,6-dinitrophenol (dinoseb; CAS No. 88-85-7) manifested testicular toxicity, and 4,6-dinitro-*o*-cresol (DNOC; CAS No. 534-52-1) showed similar but weaker testicular toxicity in laboratory animals. Consecutive doses of dinoseb and DNOC by gavage seemed to induce spermatotoxicity by disturbing spermiogenesis or the maturation process of sperm in the epididymis, and the most probable target cells of spermatotoxicity were thought to be testicular spermatids in rats. Prolonged exposure to dinoseb and DNOC in the diet also induced testicular toxicity in rats. However, the feeding dose of dinoseb irreversibly affected the early stage of spermatogenesis and produced infertility in rats. On the other hand, 2,4-dinitrophenol (DNP; CAS No. 51-28-5) did not show testicular toxicity in laboratory animals according to available literature. Further studies in laboratory animals with nitrophenolic compounds are required for clarification of their testicular toxicity and for risk assessment in humans.

Contents

1. Introduction

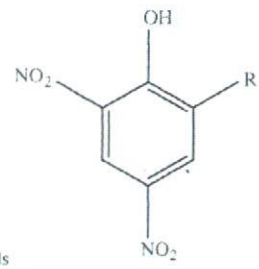
Dinitrophenolic compounds have many uses in agriculture as herbicides, insecticides, acaricides and fungicides [1]. Minor differences in chemical structure determine their use, and several compounds have more than one use (Table 1). Although the use of dinitrophenolic compounds as pesticides was banned in many countries due to their serious toxicity [2], they are still trafficked and used in agriculture. In several countries, 2-*sec*-butyl-4,6-dinitrophenol (CAS: 88-85-7; dinoseb) and dinoseb salts are registered for use as herbicides and insecticides [2]. 4,6-Dinitro-*o*-cresol (CAS: 534-52-1; DNOC) and 2,4-dinitrophenol (CAS: 51-28-5; DNP) were once used as weight-reducing agents as well. The main current use of DNOC and DNP is in the plastic industry as an inhibitor of polymerization in styrene and vinyl aromatic compounds [3,4]. It is reported that dinoseb and DNP are high-volume chemicals with production or importation exceeding 1000 tonnes per year in Organisation for Economic Co-operation and Development (OECD) member countries [5]. The annual production of DNOC is ca. 600 tonnes, and 100–200 tonnes are used as an agrochemical. In addition, significant volumes of DNOC are still stocked around the world especially in developing countries [3].

Exposure to dinitrophenolic compounds may occur by direct contact, ingestion and inhalation for users and producers, but potential indirect exposure via the environment for consumers is

* Corresponding author. Tel.: +81 3 3700 9878; fax: +81 3 3700 1408.
E-mail address: mariko@nihs.go.jp (M. Matsumoto).

0890-6238/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2008.09.008

Table 1



Scheme of dinitrophenolic compounds

Chemical name	CAS number	R	Agricultural use
2,4-Dinitrophenol (DNP)	51-28-5	H	I
4,6-Dinitro-o-cresol (DNOC)	534-52-1	CH_3	A, F, H, I
2-*sec*-Butyl-4,6-dinitrophenol (Dinoseb)	88-85-7	$CH(CH_3)CH_2CH_3$	H, I

A: acaricide; F: fungicide; H: herbicide; I: insecticide.

also anticipated. Dinoseb, DNOC and DNP have been detected in groundwater [3,4,6], and DNOC has been identified in extracts of rain and snow [3]. The use of DNP and DNOC as diet pills was discontinued in the US by the end of 1938 [4]; however, DNP was introduced in a bodybuilding magazine in the late 1990s [7], and it has managed to steadily gain popularity in some bodybuilders and athletes to rapidly lose body fat. The average daily dose of DNP or DNOC for man was about 3 mg/kg bw/day in 1930s [3,8], and the current average intake of DNP seems to be 200–400 mg/day according to a commercial web site [9].

Most dinitrophenolic compounds are absorbed well by the skin, gastrointestinal tract or lung. Dinitrophenolic compounds shows moderate to strong acute oral toxicity with LD_{50} values in the range of 25–46 mg/kg bw (dinoseb), 26–34 mg/kg bw (DNOC) and 50–71 mg/kg bw (DNP) in rats [1,10]. The major systems prone to toxicity are the hepatic, renal and nervous systems [11]. The basic mechanism of toxicity of nitrophenolic compounds is thought to be stimulation of oxidative metabolism in cell mitochondria by the uncoupling of oxidative phosphorylation [12]. Early symptoms include hyperthermia, sweating, headache and confusion. Severe exposure may result in restlessness, seizures, coma and death [11–13].

In the previous review, we showed that the administration of dinoseb to maternal animals produced developmental toxicity including teratogenicity [14]. We also reported that rats dosed with dinoseb by gavage for a total of 42 days decreased sperm motility and increased the rates of abnormal sperm, tails and heads at 7.0 mg/kg bw/day [15]. Sperm parameters are considered to be sensitive indicators of fertility [16]. In fact, the feeding dose of dinoseb to male rats for 11 weeks induced low fertility at 225 ppm (15.6 mg/kg bw/day) and infertility at 300 ppm (22.2 mg/kg bw/day) due to low or no spermatogenesis [17].

In Sertoli-germ cell co-cultures, dinoseb caused degenerative alterations in pre-pachytene and pachytene spermatocytes and Sertoli cells, while DNOC and DNP affected pre-pachytene and pachytene spermatocytes [18]. These findings suggested the possibility that dinitrophenolic compounds can produce testicular toxicity with a similar mode of action in laboratory animals and humans. However, a recent study by Takahashi et al. [19] reported no testicular toxicity in Crl:CD(SD) rats treated with DNP by gavage up to 30 mg/kg bw/day for 46 days. There were differences in testicular toxicity between dinoseb and DNP although they showed similar toxicity in Sertoli-germ cell co-cultures. In this review, we attempt to clarify the testicular toxicity of dinitrophenolic compounds in laboratory animals, and possible mechanisms involved in the testicular toxicity are discussed.

2. Testicular toxicity of dinitrophenolic compounds

The available literature for dinitrophenolic compounds shown in Table 1 was searched and reviewed for male reproductive toxicity. Only statistically significant effects are summarized below unless noted otherwise.

2.1. Testicular toxicity of dinoseb

Table 2 shows a summary of the testicular toxicity of dinoseb. When male Crj:CD(SD)IGS rats were administered dinoseb (0, 0.78, 2.33, or 7.0 mg/kg bw/day) by gavage for 42 days, motile sperm rate, progressive sperm rate, straight line velocity and viability rate were lower than the controls, and the amplitude of lateral head displacement, abnormal sperm rate and abnormal tail rate were higher than the controls at both the end of the administration and 14-day recovery periods at 7.0 mg/kg bw/day [17]. However, there were no dose related effects on spermatogenesis at the stages of spermatogonia and spermatocytes in this study.

Sato et al. [20] showed that 3-consecutive-day administration of dinoseb by gavage at 7.5 mg/kg bw/day reduced number of sperm, sperm motility, path velocity, curvilinear velocity, and amplitude of lateral head displacement in Crj:CD(SD)IGS rats. These effects were observed 19 days after the last administration, but not 12 days after the last administration. Similarly, another spermatotoxicity study, in which male Jcl:SD rats were treated with dinoseb for 5 days at 7.5 mg/kg bw/day, showed no effects on sperm parameters in the cauda epididymis 3 days after the final dosing, but reduced sperm motility and increased incidence of tailless sperm were noted 14 days after the final dosing [21]. No testicular histopathological alteration was observed in this study. These findings suggest that consecutive dose of dinoseb by gavage affects sperm by disturbing spermiogenesis or maturation processes of sperm in the epididymis in rats.

Sato et al. [20] also demonstrated that gavage dose of dinoseb at 7.5 mg/kg bw/day three times per week (Monday/Wednesday/Friday) for 3 weeks did not affect any sperm parameters in Crj:CD(SD)IGS rats. In another study, a single oral dose of dinoseb did not cause any alteration of sperm parameters 2 and 14 days after the administration at 17.5 mg/kg bw, but five daily doses of dinoseb at 7.5 mg/kg bw/day altered sperm morphology both 3 and 13 days after the last administration and caused a decreased number of sperm and the percentage motile sperm at 13 days after the last administration in SD rats [22].

In a feeding study of dinoseb at 75, 150, 225 or 300 ppm (0, 3.8, 9.1, 15.6 or 22.2 mg/kg bw/day), sperm counts and morphologically normal sperm were decreased at 150–300 ppm after 11 weeks

Table 2
Testicular toxicities in animals given dinoseb.

Species	Dose	Route	Exposure time	Effect observed	Reference
Crj:CD(SD)IGS rat	7.0 mg/kg	Gavage	42 days	↑The amplitude of lateral head displacement, ↑abnormal sperm rate and ↑abnormal tail, ↓sperm motility, ↓progressive sperm rate, ↓straight line velocity and ↓viability	[15]
Crj:CD(SD)IGS rat	7.5 mg/kg	Gavage	3 days	↓No. of sperm, ↓motile sperm, ↓path velocity, ↓curvilinear velocity and ↓amplitude of lateral head displacement	[20]
	7.5 mg/kg	Gavage	9 doses/3 weeks	No effects on sperm	
Jcl:SD rat	7.5 mg/kg	Gavage	5 days	↓Weights of seminal vesicle, prostate and epididymis, ↓sperm motility and ↑tailless sperm	[21]
SD rat	7.5 mg/kg	Gavage	5 days	↓Sperm motility, ↓no. of sperm and ↑abnormal sperm	[22]
	17.5 mg/kg	Gavage	1 day	No effects on sperm	
Sharman rat	150 ppm (9.1 mg/kg)	Diet	71–77 days	Sperm counts, ↓morphologically normal sperm, ↑adjusted weight of seminal vesicle and ↓sperm content of caudae and vasa deferentia	[17]
	225 ppm (15.6 mg/kg)	Diet	71–77 days	Infertility, ↓adjusted weights of testis and epididymides, ↑adjusted weight of seminal vesicle, ↓sperm content of caudae and vasa deferentia, sperm counts and↓ morphologically normal sperm	
	300 ppm (22.2 mg/kg)	Diet	71–77 days	↓Adjusted weight of seminal vesicle and above observed effects (225 ppm) except ↑adjusted weight of seminal vesicle	
	300 ppm (22.2 mg/kg)	Diet	10 days	No effects	
			20 days	↓Morphologically normal sperm, ↓adjusted weights of seminal vesicles and prostate	
			30 days	↓Morphologically normal sperm	
			50 days	↓Morphologically normal sperm, ↓adjusted weights of seminal vesicles	
SD rat	125–175 ppm	Diet	25 days	↓Sperm motility	[23]
Sharman rat[a]	200 ppm	Diet	153 days	Diffuse tubular atrophy of the testes	[24]
Rat	0.05% (13.5 mg/kg)	Diet	21 days	No histopathological changes in the testis	[26]
	0.02% (5.4 mg/kg)	Diet	6 months	No histopathological changes in the testis	
CD (SD) rat[a]	1–10 mg/kg	Diet	3-generation	No effects on reproduction	[6,25]
CD-1 mouse[a]	1–10 mg/kg	Diet	2 years	Testicular atrophy/degeneration with hypospermatogenesis	[6,25]

[a] Only abstract or secondary literature is available.

administration in Sherman rats [17]. In addition, spermatozoa were not found in sections of the epididymides at 300 ppm. None of 5 males fed 300 ppm and only 1 of 10 males fed 225 ppm produced litters after mating with non-treated females. There was little or no remission of these effects after a 16-week recovery period. Histopathological changes to spermatogonia, spermatocytes, spermatids and sperm in the testes were observed after 20 or 30 days administration at 300 ppm, and severe damage to the spermatogonia was observed after 50 days treatment at 300 ppm.

Similarly, a feeding dose of dinoseb (0, 125, 150 or 175 ppm) to male SD rats for 25 days showed reduced sperm motility in all treatment groups [23]. Hall et al. [24] provide a brief summary of a subchronic feeding study of dinoseb, in which Sherman rats were fed a diet with 0, 50, 100, 150, 200, 300, 400 or 500 ppm of dinoseb for 153 days. Mortality was observed at 300, 400 and 500 ppm and only animals treated with dinoseb up to 200 ppm were evaluated. Diffuse tubular atrophy of the testes was observed particularly at 200 ppm. No further details were available for this study.

The findings in the feeding dose studies suggest that prolonged exposure to dinoseb in feed affects the early stage of spermatogenesis in rats. Consistency was confirmed in a 2-year feeding study in CD-1 mice. Testicular atrophy or degeneration with hypospermatogenesis was observed at 1, 3, or 10 mg/kg bw/day [6,25]. However, a three-generation feeding study showed no effects on male reproductive toxicity in SD(CD) rats given dinoseb at 1, 3, and 10 mg/kg bw/day for 29 weeks [6,25]. An old study showed no histopathological changes in the testes of rats fed dinoseb in the diet for 21 days at 0.05% (13.5 mg/kg bw/day) or for 6 months at 0.02% (5.4 mg/kg bw/day) [26]. No further information was available.

2.2. Testicular toxicity of DNOC

A summary of testicular toxicity of DNOC is shown in Table 3. DNOC was administrated to Jcl:SD rats by gavage at 0, 4, 7.5 or 15 mg/kg bw/day for 5 consecutive days [21]. Examination at 3 days after the last dosing revealed no treatment-related alterations in histopathology of the testis and in sperm parameters. DNOC administration resulted in reduced sperm motility and increased the incidence of tailless sperm in the cauda epididymis, but there were no testicular histopathological alterations at 14 days after the last

Table 3
Testicular toxicities in animals given DNOC.

Species	Dose	Route	Exposure time	Effect observed	Reference
Jcl:SD rat	15 mg/kg	Gavage	5 days	↓Motile sperm and ↓morphologically normal sperm	[21]
Jcl:SD rat	10 mg/kg	Gavage	5 days	↑Peeled sperm	[27]
	15 mg/kg			↑Tailless sperm	
Riv:Tox(M)Wistar rat	20 mg/kg	Diet	90 days	↓Spermatogenesis and ↓relative weights of testes and prostate	[29]
Rat	0.10% (27 mg/kg)	Diet	6 months	No histopathological changes in the testis	[26]
SD(CD) rat[a]	7.20 –10.1 mg/kg	Diet	2 generations	No effects on reproduction	[3]
(C3H × C57BL/6) F1 mouse	3–12 mg/kg	Gavage	5 days	No effects on testes weight, sperm count or sperm abnormalities	[28]
(C3H × C57BL/6) F1 mouse	3–12 mg/kg	i.p.	5 days	No effects on testes weight, sperm count or sperm abnormalities	
CFLP mouse	10 mg/kg	i.p.	Single dose	↑Dominant lethal value and damage in the germ cells	[30]

[a] Only abstract or secondary literature is available.

dosing. These results indicate that consecutive gavage dose of DNOC can also affect sperm by disturbing spermiogenesis or maturation process of sperm in the epididymis in rats.

Subsequently, Takahashi et al. [27] showed that the target cells of DNOC spermatotoxicity are likely to be testicular spermatids. DNOC was administered to Jcl:SD rats by gavage at 0, 10 or 15 mg/kg bw/day for 5 days. One day after the last dosing, there were elongated spermatids that looked normal but lacked the mitochondrial sheath at the proximal end of the middle piece. Fourteen days after the last dosing, there were increases in the number of peeled sperm at 10–15 mg/kg bw/day and the number of tailless sperm at 15 mg/kg bw/day. The authors suggest that the elongated spermatids may develop into tailless sperm when they reach the cauda epididymis. In the preceding study [15], dinoseb did not affect spermatogonia and spermatocytes, but the number of abnormal sperm was increased. Therefore, spermatids may also be the target cells of dinoseb spermatotoxicity.

On the other hand, no effects were found on sperm morphology, sperm counts or testicular weights at 30 days after the last administration in (C3H × C57BL/6) F1 mice given DNOC by gavage or by intraperitoneal injection at 3–12 mg/kg bw/day for 5 days [28]. These inconsistent results may be due to the length of the post-administration period. There is a possibility that dose-related effects on spermatids, the most probable target cells, cannot be detected at 30 days after the last dosing of DNOC. Another possibility is a difference in excretion, which means that DNOC is excreted at a slower rate in rats than in mice [3].

Spermatogenesis and relative organ weights of the testes and prostate were decreased in a 90-day feeding study, in which Riv:Tox(M)Wistar rats were exposed to 20 mg/kg bw/day DNOC in the diet [29]. These findings suggest that prolonged exposure to DNOC in the diet can also induce testicular toxicity. However, an old study showed no histopathological changes in the testes in rats fed DNOC in the diet at 0.10% (27 mg/kg bw/day) for 6 months [26]. No further detailed information was available.

Intraperitoneal injection of 10 mg/kg bw DNOC into male CFLP mice increased the frequency of chromosomal aberrations in male germinal cells 20 days after administration [30]. DNOC-treated males were mated with untreated nulliparous females for 8 weeks after the treatment. New females were caged with the males every week. The number of living embryos was decreased with a dominant lethal value of 16% at the sixth week, indicating DNOC possibly affect the survival of offspring in mice. However, a two-generation feeding study revealed that there were no effects on reproduction in SD(CD) rats given DNOC at 7.20-10.1 mg/kg bw/day [3].

2.3. Testicular toxicity of DNP

Table 4 shows a summary of testicular toxicity of DNP. Unlike dinoseb and DNOC, DNP dose studies did not show testicular toxicity in rats. Crl:CD(SD)IGS rats treated with DNP by gavage at 80 mg/kg bw/day for 28 days showed increased relative organ weight of the testes, but histopathological changes in the testes, epididymis and prostate were not observed. The number of sperm in the epididymis was not affected [31,32]. Therefore, the increased relative organ weight of the testes was considered to be due to reduced body weight on the day of scheduled killing.

DNP was administrated to Jcl:SD rats by gavage at 0, 7.5, 15 or 30 mg/kg bw/day for 5 consecutive days [21]. Organ weights of the testes, epididymis, seminal vesicles and prostate were not affected at 3 days after the last dosing. Tailless sperm in the cauda epididymis was only increased slightly at 14 days after the last dosing, but there were no statistically significant effects. Similarly, organ weights of the testes and epididymis and numbers of Sertoli cells and of germ cells per Sertoli cell were not affected in Crl:CD(SD) rats treated by gavage with DNP at a dose of 0, 3, 10 or 30 mg/kg bw/day for a total of 46 days [19]. These findings indicate that a gavage dose of DNP was unlikely to possess testicular toxicity in rats under these test conditions.

Negative results were also observed in capsule and feeding dose studies. No gross or histological evidence of treatment-related testicular damage was reported following DNP treatment of dogs exposed to 5 or 10 mg/kg bw/day by capsules 6 days a week for 6 months [8] or rats exposed to 0.10% (27 mg/kg bw/day) in the diet for 6 months [26]. Small testes and testicular atrophy were observed in rats given 0.20% DNP (54 mg/kg bw/day) in the diet for 24 days. However, the authors stated that it was difficult to distinguish between direct toxic effects and secondary effects due to reduced body weight gain.

3. Discussion and conclusion

Consecutive gavage doses of dinoseb manifested testicular toxicity in rats at 7.0–7.5 mg/kg bw/day, and DNOC showed similar but weaker testicular toxicity in rats at 10–15 mg/kg bw/day. Consecutive gavage dose of DNP showed no testicular toxicity up to 80 mg/kg bw/day in rats. Consecutive doses of dinoseb and DNOC by gavage seemed to affect sperm by disturbing the spermiogenesis or maturation processes of sperm in the epididymis, and the most probable target cells of toxicity were thought to be testicular spermatids in rats.

Prolonged exposure to dinoseb and DNOC in the diet also induced testicular toxicity in rats at 9.1–22.2 and 20 mg/kg bw/day, respectively. However, a feeding dose of dinoseb irreversibly affected the early stage of spermatogenesis and produced infertility in rats at 22.2 mg/kg bw/day. In a 2-year feeding study in mice, testicular atrophy or degeneration with hypospermatogenesis was observed at 1–10 mg dinoseb/(kg bw day). However, male reproductive toxicity was not affected in rats up to 10 mg/kg bw/day for 29 weeks (three generations). No treatment-related testicular damage was reported following DNP treatment at 27 mg/kg bw/day in the diet for 6 months in rats.

In Sertoli-germ cell co-cultures, dinoseb caused degenerative alternations in pre-pachytene and pachytene spermatocytes and Sertoli cells, while DNOC and DNP affected pre-pachytene and pachytene spermatocytes [18]. Testicular effects observed in laboratory animals are thought to be caused by the uncoupling effects rather than due to a body weight loss or body temperature increase [17]. The uncoupling effects of dinoseb are stronger than that of DNP and DNOC in mouse liver and brain cells [33]; this may explain why

Table 4
Testicular toxicities in animals given DNP.

Species	Dose	Route	Exposure time	Effect observed	Reference
Crl:CD(SD)IGS rat	80 mg/kg	Gavage	28 days	No effects on sperm or sex organs	[31,32]
SPF Crl:CD(SD) rat	30 mg/kg	Gavage	46 days	No effects on sperm or sex organs	[19]
Jcl:SD rat	30 mg/kg	Gavage	5 days	No effects on sperm or sex organs	[21]
Rat	0.10% (27 mg/kg)	Diet	6 months	No histopathological changes in the testis	[26]
	0.20% (54 mg/kg)	Diet	24 days	Small testes and testicular atrophy	
Dog	5–10 mg/kg	Capsules	6 days/week for 6 months	No histopathological changes in the testis or epididymis	[8]

strong testicular toxicity was observed in dinoseb treated animals. However, the uncoupling effects of DNP were stronger than DNOC in mouse brain cells [33]. Acute toxicities in animals tend to increase with increasing uncoupling potency; however, a good correlation between *in vivo* and *in vitro* studies was not established, indicating that other factors such as absorption, distribution, metabolism and excretion may also play a role in the toxicity.

Generally, phenols tend to be absorbed rapidly and distributed throughout the body, and excretion occurs over a period of weeks [34]. DNP is metabolized rapidly to less toxic metabolites by reduction of nitro groups to amine groups [4]. Reduction of the nitro groups is also main detoxification pathway in DNOC, but oxidation of the alkyl substituent was also noted in rats and rabbits [35]. Dinoseb is metabolized by side-chain oxidation in mice, rats and dogs, followed by nitro-reduction in rats, but reduction of the nitro group was not observed in dogs and mice [34,36,37]. There were several unknown metabolites and conjugates after the administration of dinoseb and DNOC [35,36]. Some of metabolites of dinoseb and DNOC may also play a role for the testicular toxicity of these compounds. In fact, some metabolites of dinoseb (not identified) caused toxicity in the liver, kidney, spleen and blood of rats [34].

Sperm motility was reduced by gavage dosing of dinoseb and DNOC [16,20–22], and a suggestive reduction in sperm motility was also observed after DNP administration in rats [21]. Uncoupling agents such as DNP, pentachlorophenol (PCP) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) reduced mitochondrial membrane potential by attacking the proton gradient across the inner mitochondrial membrane [38,39]. Reduced sperm motility was correlated with reduced mitochondrial membrane potential caused by uncoupling agents when glucose was not present [38,40].

Of interest, *m*-dinitrobenzene (DNB), a structurally similar compound to DNP but not an uncoupling agent, causes Sertoli cell vacuolization and germ cell apoptosis in rats [41,42] but not in hamsters [43]. An intermediate metabolite, *m*-nitrosonitrobenzene, produced toxicity in rat Sertoli-germ cell co-cultures while metabolites of DNB, *m*-nitroaniline (NA) and *m*-nitroacetanilide (NAA), do not provoke testicular toxicity [44]. Seminiferous tubules isolated from hamsters were more capable of reductively metabolizing DNB to NA and NAA than that from rats, and DNB induced ATP depletion in rat seminiferous tubules, but not in hamster tubules [45].

There have been no mechanistic studies on the testicular toxicity of dinoseb and DNOC. However, like DNB, there is a possibility that metabolic activation via reductive metabolism of a nitrogroup may be responsible for the testicular toxicity of dinoseb and DNOC in laboratory animals, although dinitrophenolic compounds and DNB showed different cytotoxicity in rat Sertoli-germ cell co-cultures [18]. Understanding the basic mechanisms involved in male germ cell toxicity is necessary step to prevent reproductive failure. Further mechanistic studies on nitrophenolic compounds and compounds with similar structures are required to clarify the testicular toxicity of these compounds. No information on male reproductive toxicity was obtained for other dinitrophenolic compounds with an alkyl substituent, but similar toxicity to dinoseb and DNOC can be expected from their structures.

DNP did not show testicular toxicity in laboratory animals according to the available literature, and possibly related mechanisms were discussed. However, the histopathology of the testes could be inadequate in these DNP studies due to shorter length of exposure. The long-duration studies with DNP were conducted on dogs in 1934 and on rats in 1948 when the standard of testicular pathology is likely to be below current standard. Therefore, further studies which meet the current standard of testicular pathology [46] must be required to clarify the testicular effects of DNP.

It is noted that the efficiency of sperm production and the epididymal spermatozoal reserves of humans are considerably lower than those of conventional animal models [47]. Working [48] also described that the human male is of relatively low fertility and thus may be at greater risk from reproductive toxicants than males of the common laboratory animal model species. Furthermore, Parker [49] commented that rodent males produce sperm in numbers that greatly exceed the minimum requirement for fertility while sperm production in human males appears to be much closer to the infertility threshold; therefore, less severe reductions in sperm counts may affect human fertility significantly. These considerations indicate that definitive animal studies of chemical compounds that are suspected to have testicular toxicity are needed to assess the risk to reproduction in humans. Further histopathological studies of the testes in laboratory animals given nitrophenolic compounds and compounds with similar structures could help us to understand the testicular toxicity of these compounds, because histopathology is acknowledged as the most sensitive endpoint for detecting testicular toxicity [46].

Conflict of interest statement

There is no conflict of interest.

Acknowledgement

This study was supported by the Ministry of Health, Labour and Welfare, Japan.

References

[1] Gasiewicz TA. Nitro compounds and related phenolic pesticides. In: Laws ER, editor. Handbook of pesticide toxicology. Academic Press Inc.; 1991. p. 1191–206.
[2] PAN. PAN (Pesticide Action Network) Pesticides Database-Pesticide Registration Status; 2006.
[3] EHC 220. Environmental health criteria 220 DINITRO-*ortho*-CRESOL; 2000 [cited March 7, 2008]; available from: http://www.intox.org/databank/documents/chemical/dnoc/ehc220.htm.
[4] ATSDR. Toxicological profile for dinitrophenols. Agency for Toxic Substances and Disease Registry; 1995.
[5] OECD. The 2004 OECD list of high production volume chemicals; 2004 [cited February 2007]; available from: http://www.oecd.org/dataoecd/55/38/33883530.pdf.
[6] Health Canada. Dinoseb; 1991 [cited August 2008]; available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/dinoseb/index-eng.php.
[7] iSteroids. DNP; 2008 [cited March 7, 2008]; available from: http://www.isteroids.com/steroids/DNP.html#.
[8] Tainter M. Di-nitrophenol studies of blood, urine, and tissues of dogs on continued medication and after acute fatal poisoning. Arch Pathol 1934;18:881–90.
[9] Buy-Clenbuterol.eu. Fat loss products; 2008 [cited April 14, 2008]; available from: http://www.buy-clenbuterol.eu/products/DNP.htm.
[10] United Nations. Inclusion of chemicals in the interim prior informed consent procedure—review of notifications of final regulatory actions to ban or severely restrict a chemical-Dinoterb; 2001; available from: http://www.pic.int/incs/ICRC3/s)/English/ICRC3.15.PDF.
[11] US EPA. Nitrophenolic and nitrocresolic herbicides [cited March 7, 2008]; available from: http://epa.gov/pesticides/safety/healthcare/handbook/Chap11.pdf.
[12] Leftwich RB, Floro JF, Neal RA, Wood AJ. Dinitrophenol poisoning: a diagnosis to consider in undiagnosed fever. South Med J 1982;75:182–4.
[13] US EPA. Chemical emergency preparedness and prevention emergency first aid treatment guide (Dinoseb:88-85-7) 2007 [cited February 26, 2007]; available from: http://yosemite.epa.gov/oswer/CeppoEHS.nsf/firstaid/88-85-7?OpenDocument.
[14] Matsumoto M, Poncipe C, Ema M. Review of developmental toxicity of nitrophenolic herbicide dinoseb, 2-*sec*-butyl-4,6-dinitrophenol. Reprod Toxicol 2008;25:327–34.
[15] Matsumoto M, Furuhashi T, Poncipe C, Ema M. Combined repeated dose and reproductive/developmental toxicity screening test of the nitrophenolic herbicide dinoseb, 2-sec-butyl-4,6-dinitrophenol, in rats. Environ Toxicol 2008;23:169–83.
[16] Perreault SD, Linder RE, Strader LF, Slott V. The value of multiple endpoint data in male reproductive toxicology: revelations in the rat. Prog Clin Biol Res 1989;302:179–92.
[17] Linder RE, Scotti TM, Svendsgaard DJ, McElroy WK, Curley A. Testicular effects of dinoseb in rats. Arch Environ Contam Toxicol 1982;11:475–85.
[18] Takahashi KL, Aoyama H, Kawashima K, Teramoto S. Effects of dinoseb 4,6-dinitro-o-cresol, and 2,4-dinitrophenol on rat Sertoli-germ cell co-cultures. Reprod Toxicol 2003;17:247–52.

[19] Takahashi M, Sunaga M, Hirata-Koizumi M, Hirose A, Kamata E, Ema M. Reproductive and developmental toxicity screening study of 2,4-dinitrophenol in rats. Environ Toxicol; Published online: 6 May 2008; available from: http://www3.interscience.wiley.com/journal/119052961/abstract.
[20] Sato M, Ohta R, Wada K, Marumo H, Shirota M, Nagao T. Utilization of a computer-assisted sperm motion analysis system to examine effects of dinoseb on rat sperm. J Reprod Dev 2000;46:279–86.
[21] Takahashi KL, Hojo H, Aoyama H, Teramoto S. Comparative studies on the spermatotoxic effects of dinoseb and its structurally related chemicals. Reprod Toxicol 2004;18:581–8.
[22] Linder RE, Strader LF, Slott VL, Suarez JD. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 1992;6:491–505.
[23] Linder RE, Strader LF, McElroy WK. Measurement of epididymal sperm motility as a test variable in the rat. Bull Environ Contam Toxicol 1986;36:317–24.
[24] Hall L, Linder R, Scotti T, Bruce R, Moseman R, Heiderscheit T, et al. Subchronic and reproductive toxicity of dinoseb. Toxicol Appl Pharmacol 1978;45:235.
[25] US EPA. Dinoseb (CASRN 88-85-7). Integrated Risk Information System (IRIS); 2003 [cited August 3, 2007]; available from: http://www.epa.gov/iris/subst/0047.htm.
[26] Spencer H, Rowe V, Irish D. Toxicological studies on laboratory animals of certain alkyldinitrophenols used in agriculture. J Ind Hyg Toxicol 1948;30:10–25.
[27] Takahashi KL, Takahashi N, Hojo H, Kuwahara M, Aoyama H, Teramoto S. Pathogenetic transition in the morphology of abnormal sperm in the testes and the caput, corpus, and cauda epididymides of male rats after treatment with 4,6-dinitro-o-cresol. Reprod Toxicol 2006;22:501–7.
[28] Quinto I, De Marinis E, Mallardo M, Arcucci A, Della Morte R, Staiano N. Effect of DNOC Ferbam and Imidan exposure on mouse sperm morphology. Mutat Res 1989;224:405–8.
[29] den Tonkelaar EM, Van Leeuwen FXR, Kuiper C. Semichronic toxicity testing of DNOC in the rat. Med Fac Landbouww Rijksuniv Gent 1983;48:1015–22.
[30] Nehez M, Selypes A, Paldy A, Berencsi G. The mutagenic effect of a dinitro-o-cresol-containing pesticide on mice germ cells. Ecotoxicol Environ Saf 1978;2:401–5.
[31] Koizumi M, Yamamoto Y, Ito Y, Takano M, Enami T, Kamata E, et al. Comparative study of toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats. J Toxicol Sci 2001;26:299–311.
[32] MHLW, Japan. Twenty-eight-day repeat dose oral toxicity test of 2,4-dinitrophenol in rats. Toxicity testing reports of environmental chemicals, vol. 8. Ministry of Health, Labour and Welfare, Japan; 2001. p. 7–36.
[33] Ilivicky J, Casida JE. Uncoupling action of 2,4-dinitrophenols 2-trifluoromethylbenzimidazoles and certain other pesticide chemicals upon mitochondria from different sources and its relation to toxicity. Biochem Pharmacol 1969;18:1389–401.
[34] Kaufman DD. Phenols. In: Kearney PC, Kaufman DD, editors. Herbicides: chemistry, degradation and mode of action. New York: Marcel Dekker Inc.; 1976. p. 665–707.
[35] Leegwater DC, van der Greef J, Bos KD. Integrated studies on the metabolic fate of DNOC. II. Biotransformation in mammals. Med Fac Landbouww Rijksuniv Gent 1982;47:401–8.
[36] Bandal SK, Casida JE. Metabolism and photoalteration of 2-*sec*-butyl-4,6-dinitrophenol (DNBP herbicide) and its isopropyl carbonate derivative (dinobuton acaricide). J Agric Food Chem 1972;20:1235–45.
[37] FAO, WHO. The monographs (Binapacryl); 1970 [cited April 16, 2008]; available from: http://www.inchem.org/documents/jmpr/jmpmono/v069pr02.htm.
[38] Gravance CG, Garner DL, Miller MG, Berger T. Flow cytometric assessment of changes in rat sperm mitochondrial function after treatment with pentachlorophenol. Toxicol In Vitro 2003;17:253–7.
[39] Linsinger G, Wilhelm S, Wagner H, Hacker G. Uncouplers of oxidative phosphorylation can enhance a Fas death signal. Mol Cell Biol 1999;19:3299–311.
[40] Mukai C, Okuno M. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. Biol Reprod 2004;71:540–7.
[41] Strandgaard C, Miller MG. Germ cell apoptosis in rat testis after administration of 1,3-dinitrobenzene. Reprod Toxicol 1998;12:97–103.
[42] Blackburn DM, Gray AJ, Lloyd SC, Sheard CM, Foster PM. A comparison of the effects of the three isomers of dinitrobenzene on the testis in the rat. Toxicol Appl Pharmacol 1988;92:54–64.
[43] Obasaju MF, Katz DF, Miller MG. Species differences in susceptibility to 1,3-dinitrobenzene-induced testicular toxicity and methemoglobinemia. Fundam Appl Toxicol 1991;16:257–66.
[44] Cave DA, Foster PM. Modulation of *m*-dinitrobenzene and *m*-nitrosonitrobenzene toxicity in rat Sertoli-germ cell cocultures. Fundam Appl Toxicol 1990;14:199–207.
[45] Jacobson CF, Miller MG. Species difference in 1,3-dinitrobenzene testicular toxicity: in vitro correlation with glutathione status. Reprod Toxicol 1998;12:49–56.
[46] Lynda LL, Dianne MC, Robert EC, Peter CM, Norman JB, Karen SR, et al. Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicol Pathol 2002:507–20.
[47] Amann RF. A critical review of methods for evaluation of spermatogenesis from seminal characteristics. J Androl 1981;2:37–58.
[48] Working PK. Male reproductive toxicology: comparison of the human to animal models. Environ Health Perspect 1988;77:37–44.
[49] Parker RM. Testing for reproductive toxicity. In: Hood RD, editor. Developmental and reproductive toxicology. New York: CRC Press; 2006. p. 425–87.

Reproductive and Developmental Toxicity Screening Study of 2,4-Dinitrophenol in Rats

Mika Takahashi,[1] Masao Sunaga,[2] Mutsuko Hirata-Koizumi,[1] Akihiko Hirose,[1] Eiichi Kamata,[1] Makoto Ema[1]

[1]Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Tokyo 158-8501, Japan

[2]Safety Research Institute for Chemical Compounds Co., Ltd., Sapporo 004-0839, Japan

Received 7 December 2007; revised 13 March 2008; accepted 24 March 2008

ABSTRACT: Rats were treated by gavage once daily with 2,4-dinitrophenol (DNP) at 0 (control), 3, 10, or 30 mg/kg bw. Males were dosed for 46 days, beginning 14 days before mating, and females were dosed for 40–47 days, from 14 days before mating to day 3 of lactation. No deaths were observed in males and females of any group. A significant decrease in body weight gain and significant increase in liver weight were found in males and females at 30 mg/kg bw/day. The number of live pups on postnatal days (PNDs) 0 and 4, live birth index, and body weight of live male and female pups on PNDs 0 and 1 were significantly lowered at 30 mg/kg bw/day. External and internal examinations of pups revealed no increased incidence of malformations in DNP-treated groups. On the basis of these findings, we concluded that DNP has general and reproductive/developmental toxicity, but not teratogenicity, under the present conditions. The NOAEL of DNP is considered to be 10 mg/kg bw/day in rats. © 2008 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2008.

Keywords: 2,4-dinitrophenol; reproductive/developmental toxicity; rat

INTRODUCTION

2,4-Dinitrophenol (DNP; CAS No. 51-28-5) is one of the six different isomers of dinitrophenols, and the most commercially important isomer. Commercial dinitrophenol, a mixture of DNP and smaller amounts of 2,3- and 2,6-dinitrophenol, is used in the synthesis of picric acid and picramic acid, and for making dyes, wood preservatives, photographic developers, explosives, and insecticides (ATSDR, 1995). The production volume of DNP exceeded 1 million pounds/year in the U.S. (Scorecard, 2007) and was around 1000 tons in Japan in 2005 (METI, 2006). DNP is used for the same purposes as dinoseb, 2-*sec*-butyl-4,6-dinitrophenol, which was registered as a herbicide and insecticide. DNP was once taken extensively as a weight reduction drug in the 1930s (Simkins, 1937a,b). Thereafter, adverse effects, including cataracts, renal damage, and death due to hyperthermia, were noted in people who took DNP (Beinhauer, 1934; Epstein and Rosenblum, 1935; Goldman and Haber, 1936; Simkins, 1937a,b). DNP was banned for use for this purpose by authorities in the U.S. in 1938 (Parascandola, 1974; Kurt et al., 1986); however, it can be still illicitly purchased in the U.S. as a diet pill via commercial web sites, and incidents, including deaths, have been reported (Miranda et al., 2006). DNP is released into the environment primarily during its manufacture and use, and from waste disposal sites that contain DNP (ATSDR, 1995), and can also form in the atmosphere from the reaction of benzene with NOx in ambient air (Nojima et al., 1983). General population and occupational exposures may occur primarily through the inhalation of ambient air (ATSDR, 1995). According to TRI01 (U.S. EPA, 2001), total on- and off-site release was around 100 000 pounds in

Correspondence to: M. Ema; e-mail: ema@nihs.go.jp

Contract grant sponsor: Ministry of Health, Labor and Welfare, Japan

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.20398